

## THE EFFECTS OF RADIATION FROM URANIUM MILL TAILINGS ON *TRADESCANTIA*<sup>1</sup>

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**ABSTRACT.**— To assess the potential hazard from radioactive wastes, a plant with known radiosensitivity, *Tradescantia* clone 02, was grown in radioactive soil obtained from uranium mill tailings. The levels of radiation on these tailings varied from 0.03mR/hr (background) to a maximum of 3.00 mR/hr. *Tradescantia* grown in soil with a radiation level greater than 0.10 mR/hr evidenced significantly reduced reproductive integrity and fecundity, as measured by the number of stunted hairs per stamen, pollen viability, and numbers of somatic mutations. Based on these data, the radioactivity from uranium mill tailings has the potential to alter normal plant succession due to its detrimental effect on any species that is relatively radiosensitive.

An increase in atomic power as a source of domestic energy will lead to increased levels of ambient radiation locally. Uranium mill tailings are a case in point: there environmental contamination by various radionuclides may occur many years after a mill has shut down (Sears et al. 1975). This radioactivity may not only contaminate surrounding terrestrial and aquatic communities (Pendleton et al. 1964, Sears et al. 1975), but any species colonizing these tailings will have to contend with a radiation level-exceeding background. The radiosensitivity of colonizing species may ultimately determine plant succession patterns.

*Tradescantia* demonstrates an extreme sensitivity to ionizing radiation, i.e., it is highly radiosensitive (Sparrow, Underbrink, and Sparrow 1967, Sparrow, Underbrink and Rossi 1972). The ability of this plant to respond to differing levels of ionizing radiation and its effects can be seen in the work by Mericle and Mericle (1965a,b), Ichikawa and Sparrow (1968), and Underbrink, Schairer, and Sparrow (1973). In this study, *Tradescantia* was used to evaluate potential effects of low-level chronic radiation on plant succession. The specific hypothesis to be tested was: levels of ionizing radiation emitted from uranium mill tailings have the

potential to increase somatic mutations and to decrease pollen viability and overall reproductive integrity of radiosensitive species colonizing the tailings.

### MATERIALS AND METHODS

Soil samples from five sites on the Vitro Chemical Plant (located at 3300 South 900 West, Salt Lake City, Utah) mill tailings were collected in 1975. The levels of activity at the sites from where our soil samples were taken were 1.11, 1.62, 2.04, 2.72, and 3.32 mR/hr with the latter representing the highest radiation level detected on the tailings. The control had an activity of 0.01 mR/hr. After transporting the tailings from the Vitro Mill site to the greenhouse, the amount of ionizing radiation was measured using a Victoreen Thyac III survey meter in the absence of the Vitro radiation field. The new levels were 0.07, 0.10, 0.14, 0.20, and 0.19 mR/hr, respectively, with the control remaining at 0.01mR/hr.

*Tradescantia*, clone 02 (obtained from A. H. Sparrow, Brookhaven National Laboratory), was used as the test species. Clone 02 is a perennial, herbaceous, diploid plant ( $2n=12$ ), with narrow tapering leaves (not unlike some medium-sized grasses), and

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reaches a height of about 60 cm. It is heterozygous for blue and pink flower color and is thought to be a hybrid between *T. occidentalis* and *T. ohiensis* (Sparrow et al. 1961).

Thirty-six plants of equal size and containing young inflorescences were selected from a stock population. Root systems were washed in water to remove soil. Each treatment consisted of six plants repotted in either radioactive tailings from one of the five sites or the control soil. Plants were grown under greenhouse conditions with a 16-hr light cycle, a day temperature of 35 C, and a night temperature of 20 C. The pH of all radioactive tailings was adjusted to that of the control soil (pH 8.0) with  $\text{CaCO}_3$ . A nutrient solution was added to each pot once every five days through the first two weeks (Blankedaal et al. 1972). Plants at each radiation level were separated by 30 cm on the same greenhouse bench, and those in the control soil were located in an adjacent room.

Ten days after the plants had been potted, data were collected for 20 continuous days on the number of stunted hairs, somatic mutations per stamen, and percent pollen viability. The 10-day lag period served two purposes: 1) it allowed the plants to recover from the shock of transplanting, and 2) effects of radiation treatment are normally not seen for one week to 10 days (Ichikawa and Sparrow 1967, Underbrink et al. 1970). A maximum of four flowers were chosen randomly from each treatment group each day, and from each of these flowers one stamen was chosen at random for scoring. An arbitrarily chosen antipetalous stamen was numbered as one, with the next stamen in a clockwise direction being two, and the next three. The antisepalous stamen located between stamen one and two is designated four, with five and six located in a similar clockwise manner. The selected stamen was then divided into three arbitrary regions for ease of scoring. The types of somatic aberrations scored were blue to pink, blue to colorless, pink to colorless, colorless to pink, normal size cell to giant or dwarf, and branched hairs (Underbrink et al. 1973). Loss of reproductive integrity in stamen hairs was measured by the number of stun-

ted hairs (Ichikawa and Sparrow 1967, 1968). An average hair from a healthy plant consists of 20.5 cells, and a stunted hair has been defined as one with 12 cells or less (Ichikawa and Sparrow 1967, Underbrink et al. 1973).

Reproductive integrity is the ability of somatic tissue to achieve the number of mitotic cycles characteristic of the species that lead to its natural size and shape. The number of stunted hairs has been correlated with reproductive integrity by demonstrating that these hairs do not obtain their normal length due to a slowing or complete cessation of the mitotic cycle (Ichikawa and Sparrow 1968). Pollen viability was determined by randomly selecting two of the four flowers previously picked and smearing five anthers on a slide to release the pollen grains. The pollen was then stained with acetocarmine. Viable pollen absorbs this stain and the nucleus appears red; aborted pollen remains yellow. Data were recorded as percent viable and nonviable pollen. Each parameter was subjected to an analysis of variance using a completely randomized design followed by statistical comparisons for locating significant differences among the treatments.

#### Radiation source analysis

Nayar, George, and Gopan-Ayengar (1970) have shown that the ionizations from radionuclides in radioactive monazite sand that were absorbed by roots of *Tradescantia* were more effective than external radiation in increasing the frequency of somatic mutations. A similar experiment was conducted with a tailings sample from the Vitro Mill. The purpose was to determine whether differences in the number of somatic mutations per stamen, stunted hairs per stamen, and pollen viability were due to internally absorbed radionuclides or to external ionizations.

Six plants of equal size and containing young inflorescences were potted in soil with a background radiation level of 0.01 mR/hr. A plastic bag was placed around each plot and all six arranged in a small wooden enclosure. Mill tailings from the 0.19 mR/hr treatment were then evenly dis-

tributed around the six plants. In both experiments, the treated plants were exposed to equal amounts of radioactive tailings. Six control plants were potted in soil with a background radiation level of 0.01 mR/hr and located in an adjacent room. All plants were grown in a glasshouse. Ten days after potting, data were collected on mutations per stamen, stunted hairs per stamen, and percent pollen viability for 15 consecutive days in a manner similar to that described above. The data were analyzed with Student's t-test.

RESULTS

Average numbers of mutations per stamen for each level of radiation are presented in Figure 1. An analysis of variance (ANOVA) revealed significant differences between treatments; statistical comparisons revealed where the differences occurred. Three subsets appeared within the six radiation treatments. A linear regression analysis with adjusted treatment means demonstrated a significant correlation, ( $r=.78$  and  $r^2=.62$ ) between radiation level and number of mutations per stamen.

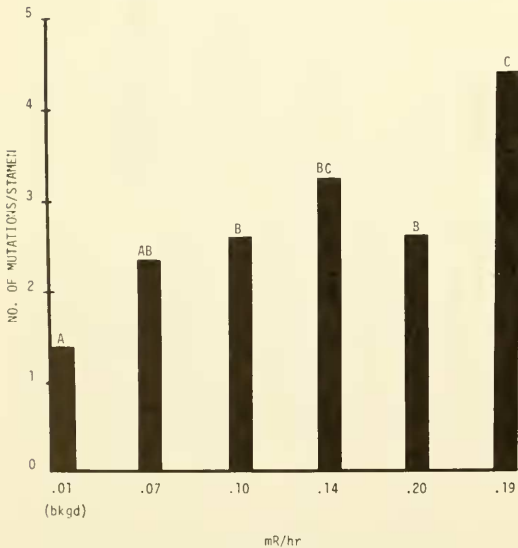


Fig. 1. Mutations per stamen in *Tradescantia* clone 02, as affected by ionizing radiation from radioactive mill tailings. Columns with the same letter above are not significantly different.

Figure 2 shows the average number of stunted hairs per stamen for each treatment. Again, a significant difference was shown by the ANOVA test. Three major subsets were apparent, with different treatments appearing in more than one subset. A linear regression, using adjusted means, indicated that a significant correlation ( $r=.88$  and  $r^2=.77$ ) existed between radiation level and number of stunted hairs per stamen.

Pollen viability ratings, averaged for each treatment, are shown in Figure 3. Analysis of variance identified a significant difference between the different radiation levels. The statistical comparisons grouped the six treatments again into three subsets. There was some overlapping of subsets among the treatments. The linear regression of radiation level versus pollen viability was not significant.

The results from the radiation source analysis experiment are presented in Table 1. There were no significant differences between the control and treated plants in the number of mutations per stamen, stunted hairs per stamen, or pollen viability.

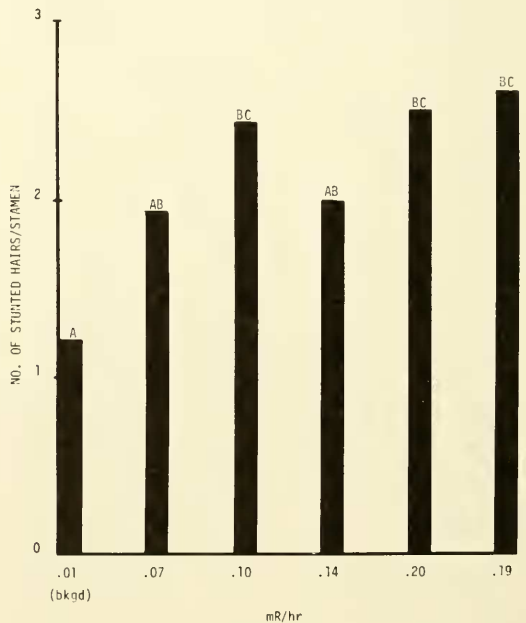


Fig. 2. Stunted hairs per stamen in *Tradescantia* clone 02, as affected by ionizing radiation from radioactive mill tailings. Columns with the same letter above are not significantly different.

## DISCUSSION

Early studies conducted by Sparrow and Pond (1956), using two *Antirrhinum* clones, demonstrated that exposure levels of 16mR/hr significantly increased mutation rates. They also suggested that much lower doses also increased mutations.

A more comprehensive analysis, correlating low-level radiation and its effect on the stamen hairs of *Tradescantia* clone 02, has been conducted by Mericle and Mericle (1963, 1965a,b). Their procedure involved exposing plants to geological dikes where the radioactivity was two to five times higher than normal. They detected significant changes in the number of somatic mutations and stunted hairs per stamen at 0.10 and 0.25 mR/hr. Since the plants were potted in normal soil this increase was attributed primarily to external radiation.

Results of this study closely paralleled those obtained by Mericle and Mericle (1963). All plants subjected to 0.10 mR/hr and above differed significantly from the control plants. The only exception was in the number of stunted hairs per stamen. Here, plants subjected to 0.10 mR/hr were statistically distinct from the controls, but plants grown in soil at 0.14 mR/hr showed no significant difference from the controls. Although there appeared to be variations throughout the six treatments in the number of mutations and stunted hairs per stamen, the trend was for an increase as radiation increased. Pollen viability, however, did not show a significant correlation with increasing radiation, although viability did decrease when the comparisons involved the two extremes (Fig. 3). In contrast to the studies by Mericle and Mericle (1965a,b), we found no disparity in the qualitative analyses of the stamen hairs or pollen from

0.10 mR/hr to the highest treatment levels (0.20 and 0.19 mR/hr). The present study also revealed that plants grown in the 0.07 mR/hr soil were similar to the control plants. Thus, the critical dosage in this study seemed to lie between 0.07 and 0.10 mR/hr.

The data from the *Tradescantia* stamen hairs and pollen support our hypothesis, but only relative to plants with a high radiosensitivity. Stated in another way, species characterized by low radioresistance, e.g., *Tradescantia* clone 02, would have to adjust to an increase in somatic mutations, and a decrease in reproductive integrity and pollen viability, when growing on the radioactive mill tailings from the Vitro Chemical Plant.

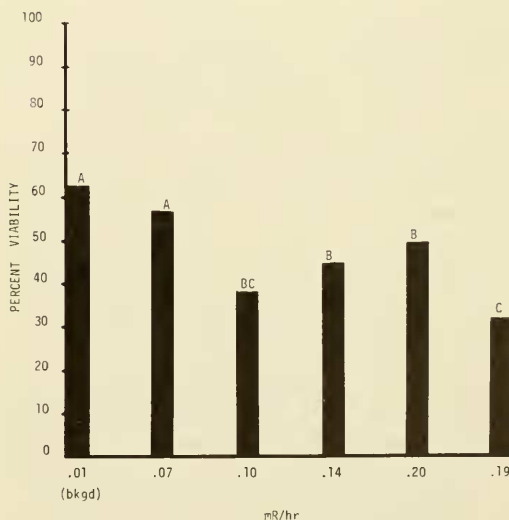


Fig. 3. Pollen viability in *Tradescantia* clone 02, affected by ionizing radiation from radioactive mill tailings. Columns with the same letter above are not significantly different.

TABLE 1. Student's t test for comparing stamen hairs and pollen from *Tradescantia* which were subjected to an external radiation dose of 0.18 mR/hr or 0.01 mR/hr.

Parameter	Averages		d.f.	t Value
	Control (0.01 mR/hr)	Treatment (0.18 mR/hr)		
Somatic mutations per stamen	2.31	2.44	68	0.19
Stunted hairs per stamen	1.42	1.32	68	0.24
Pollen viability (percent)	44.76	49.13	22	0.88



The radiation source experiment was an attempt to verify the origin of the ionizations that contributed to the change in mutation rate, reproductive integrity, and pollen viability in *Tradescantia* clone 02. Our data suggest that the source was internal. This contradicts the results of Mericle and Mericle (1965a), though absorption of radioactive radon gas through the leaves could not be eliminated as a possibility. In the present study, all internal radiation must have been a product of root absorption. Mistry et al., (1965) and D'Souza and Mistry (1970) examined the uptake of various radionuclides by plants inhabiting radioactive monazite sand and those growing in hydrosolutions. Radium-226 was transported more readily and it accumulated in higher concentrations in stems and leaves of treated plants than did any other radionuclide. Plants can, however, absorb other radionuclides such as uranium-238, thorium-230, lead-210, and polonium-210 (Dinse and LaFrance 1953, D'Souza and Mistry 1970). The ionizing radiation from the Vitro Chemical Plant mill tailings is due, in part, to these same elements, which represent the uranium decay series (Sears et al. 1975). *Tradescantia* may have absorbed radionuclides through its root system, and these nuclides may have caused the internal ionizations, with perhaps radium-226 the predominant isotope involved.

Levels of radiation measured in the greenhouse were considerably lower than those recorded on the mill tailings. If it had been possible to grow these plants on the site, the differences between treatments and controls might have been more pronounced (Nayer and Sparrow 1967, Ichikawa 1971). The decrease in ionizing radiation may have been caused by a decline in the amount of radioactive radon gas, a member of the uranium decay series. Radon gas is reportedly trapped in the soil layers by any mechanism that tends to seal the surface (Osburn 1965), e.g., a lack of vegetation or very little precipitation. Pendleton (pers. comm.), however, has indicated that radon gas is very poorly trapped in soil layers. Transferring the tailings to the glasshouse disturbed the layers, thus the radon gas may have been released resulting in a decrease in activity.

A second probable cause was that only small volumes of soil were sampled in the glasshouse whereas large volume of soil contributed ionizing particles at the Vitro Mill site.

Radiological surveys taken on the five radioactive soils as they existed in the greenhouse revealed that the two samples with the most radiation were approximately equal in activity, whereas in the field there was a much larger difference. Reasons for the initial gross decrease have already been discussed. Subtle similarities and variations are related to the Vitro Chemical Plant and its milling operations. First, ore for the mill was appropriated from numerous mines and is thus composed of several different mineral types. Second, the procedure for extracting the uranium was altered in 1956; thus the soil texture and its chemical makeup varied from one site to another. This affects the presence and availability of numerous radionuclides via differential leaching rates on different soil types. Consequently, the radioactivity in soil samples will vary, and the degree to which radionuclides are available to the plants will differ (Osburn 1965, Prokhorov 1973). These factors help explain the similarity between the two with the most radiation.

Although the radiation levels from the tailings of the Vitro Chemical Plant caused detrimental effects in *Tradescantia* stamen hairs, one must realize that not all plant species exhibit this degree of radio-sensitivity, especially when the stamen hairs themselves are more sensitive to radiation than the rest of the plant (Sparrow and Schwemmer 1974). A species may possess one of many physiological and morphological attributes that will allow it to exist in an environment subjected to above normal radiation levels, e.g., nutritional state, growth hormone concentrations, plant size, and depth of roots, time requirement for mitosis and meiosis, percentage of cells dividing in the meristem, and, most importantly, interphase chromosome volume (ICV) (Gunken and Sparrow 1961). However, radioactive uranium mill tailings, which represent a source of low-level chronic radiation, are a potential hazard to any species trying to establish on these soils.

If the species has a relatively high radioresistance, it will not be affected by the radiation. The other possibilities are a lower fecundity, a higher somatic mutation rate, and a decrease in reproductive integrity. These three aspects, working together, would decrease the competitive ability of a radiosensitive population and thus alter its chances of becoming successfully established. Only the more radioresistant species will be able to colonize such sites. Succession on radioactive uranium mill tailings, over a long period, therefore, may not proceed in the same direction or at the same rate as on a similar area lacking radiation stress. And with low-level radiation, the change will not be apparent until succession has proceeded to the point where the natural turnover time in species composition is greater than the time required for radioselection to be efficient.

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